

# Double Etched Porous Silicon Films for Improved Optical Sensing of Bacteria

M. B. Gongalsky,<sup>2</sup> A. A. Koval, S. N. Schevchenko, K. P. Tamarov, and L. A. Osminkina

Faculty of Physics, Moscow Lomonosov State University, Moscow 119991, Russia

Combination of conventional electrochemical etching and subsequent metal-assisted chemical etching (MACE) was used to obtain double etched porous silicon (DEPSi) structures with good optical properties and high affinity to bacteria. Second etching step increased the roughness of the surface and created more cavities for entrapping of bacteria, while optical scattering on the surface was still insufficient and quality of infrared Fabry-Perot interferograms remained high. Obtained DEPSi structures were used for detection of bacteria (E. Coli). Fast Fourier transform of infrared spectra showed reversible broadening of the main band, what allows to detect bacteria in concentrations down to 10<sup>4</sup> CFU per mL.

© The Author(s) 2017. Published by ECS. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/2.1821712jes] All rights reserved.

Manuscript submitted April 18, 2017; revised manuscript received August 14, 2017. Published September 16, 2017.

Development of new materials for advanced sensing of viruses and bacteria is a very intriguing direction of modern bioengineering today. Rapid detection of microorganisms or various biomolecules is required for medicine and food industry in order to control air and water safety or to perform express clinical tests. On contrary conventional testing is based on time-consuming laboratory analysis, which sometimes requires highly trained personnel.

The most common sensors are in fact electronic or optical devices. They may include nanostructures such as silicon nanowires,<sup>1</sup> carbon nanotubes<sup>2</sup> and graphene,<sup>3</sup> which provide better sensitivity due to high surface area. One of the most promising nanomaterials is porous silicon (PSi),<sup>4</sup> because of its unusually high porosity,<sup>5</sup> pore morphology,<sup>6</sup> photoluminescence in visible and near infrared range of spectrum, simple fabrication procedure, etc. In fact its specific area as high as 800 m<sup>2</sup>/cm<sup>3</sup> makes it extremely sensitive to an environment.<sup>7</sup>

There are some optical sensors based on PSi.<sup>8</sup> The simplest way to detect bacteria is to measure reflectivity deviation of analyte under laser irradiation.<sup>9,10</sup> The sensitivity of 10<sup>3</sup> colonial-forming units (CFU) per mL could be reached,<sup>9</sup> but sophisticated antimicrobial peptide functionalization is required. Another idea is to modify reflection pattern created by microarray of holes in Si wafer, which acts as a grating sensitive to the bacteria inside the holes.<sup>11,12</sup> No additional functionalization was used, but sensitivity was about 10<sup>5</sup> CFU per mL only.

However the most promising approach includes interferometry, e.g. using microcavities in PSi photonic crystal filled with grampositive or gram-negative bacteria.<sup>13</sup> Similar sensors were also successfully employed to detect DNA<sup>14</sup> and immunoglobulin IgG.<sup>15</sup> Improved accuracy can be achieved by fast Fourier transform (FFT) of interferogram aimed to precise calculation of effective optical thickness of PSi photonic crystal.<sup>5</sup> Moreover double-layered sensing structures with different porosity were employed to form beat-like interferogram, where FFT spectrum had two distinguishable peaks.<sup>16</sup>

Although several interferometric sensor prototypes have been proposed, the issue of efficient adhesion of bacteria on PSi surface is still relevant. That can be crucial parameter for both sensitivity and performance of the sensor, especially if it operates in flow mode. One solution is conjugation of PSi surface with special molecules highly affinitive to bacteria, but it makes the sensor less stable and more expensive. Another option is modification of PSi surface itself, but there is always a trade in between roughness of the surface and optical quality of the interferometer. Here we propose mild modification of mesoporous silicon (MPSi) surface by metal-assisted chemical etching (MACE), which results in thin layer of porous nanowires. Thus, new array of pores from 200 nm to 2  $\mu$ m is formed between the nanowires. This double etched porous silicon (DEPSi) layer can ef-

ficiently trap bacteria on the surface, while optical quality remains good enough for interferometric measurements.

#### Experimental

MPSi was obtained by electrochemical etching of silicon monocrystaline wafers (crystallographic orientation – 100, specific resistivity – 1...5 mOhm\*cm) in a mixture of hydrofluoric acid (HF) and ethanol (1:1 by volume). Current density was 50 mA/cm<sup>2</sup> and duration of etching was 60 min.

DEPSi was formed by metal-assisted chemical etching (MACE) of MPSi. First, silver nanoparticles were deposited on the surface of MPSi by submerging the sample into a mixture (1:1 by volume) of 5M HF and silver nitrate (AgNO<sub>3</sub>) for 30 seconds. Second, the sample was submerged into a mixture of 5M HF and H<sub>2</sub>O<sub>2</sub> (30%) (1:10 by volume) for 20 min. After that hydrogen had been removed from the surface by thermal annealing of both DEPSi and MPSi in air at 350°C in order to increase hydrophilicity of the samples. Finally silver nanoparticles were removed by washing into nitric acid (HNO<sub>3</sub>, 30%) for 15 min. Note, that silver nanoparticles may demonstrate anti-bacterial activity themselves,<sup>17,18</sup> but it was shown that nitric acid cleaning provide complete dissolution of silver.<sup>19,20</sup> That allows us to use silver catalyst for biomedically oriented nanostructures. Indeed, more bioinert metals such as gold also can be used for MACE.<sup>21</sup> But the procedure is more complicated and may require lithography for successful etching.<sup>21</sup>

Scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy were employed to investigate interaction between MPSi, DEPSi and E. Coli (Escherichia Coli). The latter with concentration about  $10^5$  CFU per 1 mL were deposited from 30  $\mu$ L drop on the surface of the samples. The suspension of bacteria was filtered from culture medium via centrifugation and all the bacteria were transferred into water. Then the drop had being dried for 30 minutes. In order to check the reversibility of the experiment, the bacteria were removed by rinsing in ethanol.

### Results

Figure 1 shows SEM images of single etched MPSi and DEPSi. According to Figures 1a, 1c MPSi is a porous layer with typical nanocrystal size about 10 nm. Thickness of the MPSi layer is 56  $\mu$ m. DEPSi consists of two layers, i.e. bottom single etched MPSi layer and top double etched layer (Figures 1b, 1d). Thicknesses of bottom and top layers are about 53 and 3  $\mu$ m, respectively. Figure 1d clearly shows that bottom layer is in fact well-aligned vertical nanowires and each of them consists of mesoporous material. Top layer has higher porosity and bigger holes between nanowires.

<sup>z</sup>E-mail: mgongalsky@gmail.com

On the one hand the roughness of the surface has been significantly increased after MACE, and a lot of micrometer-scale holes



**Figure 1.** SEM images: (a) top view of MPSi, (b) top view of DEPSi, (c) side view of MPSi, (d) side view of DEPSi. Pore volume distributions for MPSi (e), DEPSi (f).

and cavities appeared according to the SEM images. Those holes are supposed to be efficient traps for bacteria on contrary with surface of MPSi, which is literally flat in micrometer scale. Since quantitative analysis of pore morphology by SEM is hindered, it was provided by low temperature nitrogen adsorption isotherms technique.

Figures 1e, 1f show pore distribution for MPSi (left plot) and DEPSi (right plot) in nanometer range. Here one can see that MACE process increases "nanoroughness" of the film as well, since average pore diameter increased from 10 to 15 nm and moreover some bigger pores in the range of 15–40 nm appeared. That opens additional possibilities for trapping of bacteria. All bacteria have huge variety of nanometer sized receptors on their membrane, which can stick to DEPSi surface in accordance with van der Waals mechanism. Similar effect has been observed for viruses bound by PSi nanoparticles in aqueous suspensions.<sup>22</sup>

Adsorption isotherms (not shown here) also give us porosity and specific area values for both materials. Specific surface area,  $S_D$ , and pore volume,  $V_D$ , in DEPSi were equal to  $(305 \pm 5) \text{ m}^2/\text{g}$ , and  $(1.20 \pm 0.03) \text{ cm}^3/\text{g}$ , respectively, which corresponds to volume porosity,  $P_D = (73 \pm 3)\%$ . Same parameters for MPSi,  $S_M$ ,  $V_M$ ,  $P_M$ , were equal to  $(265 \pm 5) \text{ m}^2/\text{g}$ ,  $(0.73 \pm 0.03) \text{ cm}^3/\text{g}$ ,  $(63 \pm 3)\%$ , respectively, which indicates once again higher porosity, roughness and sensitivity of DEPSi by comparison with MPSi.

In addition to the SEM images, which gave us good visual description of bacteria adhesion, FTIR spectroscopy could provide both characterization of the samples and quantitative integrated analysis, detection of bacteria itself. Figure 2 shows FTIR transmission spectra for as-prepared MPSi (black curve), and DEPSi (red curve). There are Si-H valence vibration bands between 2087–2140 cm<sup>-1</sup>, Si-H scissors oscillations at 906 cm<sup>-1</sup> and Si-H deformation at 624 cm<sup>-1</sup>. This points to hydrogen termination of the MPSi surface, which governs



Figure 2. FTIR spectra of MPSi (black), DEPSi (red).



Figure 3. SEM images of porous silicon with E. Coli adsorbed on the surface with different magnification.

its hydrophobic properties. Comparison between two FTIR spectra shows similarity of their optical properties.

Efficient binding between bacteria and the PSi surface was confirmed by direct observation of interaction between porous films and bacteria and by FTIR spectroscopy. Figures 3a, 3b show bacteria deposited on the porous silicon surface. FTIR transmission spectra of DEPSi before and after adhesion of the bacteria are shown on Figure 4 in order to demonstrate changes of chemical surface composition of the samples. The adhesion of the bacteria leads to appearance of C=C (double bond)  $\pi$  C=C (triple bond) valence peaks at 1600 and 2100 cm<sup>-1</sup>, respectively, which can be attributed to the bacteria.

Obtained DEPSi samples were used to create a prototype of sensor element, which schematic view is shown on Fig. 5c. The element contains silicon wafer (gray colored) with MPSi layer (orange colored) and a layer of DEPSi on MPSi layer (orange colored rods).



Figure 4. FTIR spectra of pure DEPSi (black) and DEPSi with E. Coli adsorbed on the surface (red).

A broadband infrared ray has normally fallen on the surface of the sensor (slightly inclined on the figure for illustrative purpose) and then reflected from all the surface interfaces between DEPSi, MPSi and c-Si layers. The interference of the reflected light was detected by FTIR spectrometer. The scheme shows two cases, i.e. one without bacteria (on the left of the figure) and another one with bacteria (on the right of the figure) specified by different distance between maxima



Figure 5. (a) FTIR interferogram of DEPSi before adhesion of E. Coli (black), after adhesion of E. Coli (red), and after further removal of E. Coli (blue). (b) FFT image of FTIR spectra from (a), same color encoding. (c) Schematic view of sensor element based on DEPSi. Detection without bacteria (on the left) and with bacteria (green ellipse) on the surface (on the right) is shown. Light sources are shown as violet sectors.

Downloaded on 2017-09-19 to IP 93.180.48.13 address. Redistribution subject to ECS terms of use (see ecsdl.org/site/terms\_use) unless CC License in place (see abstract).

Table I.	Porosities and	refractive ind	lexes of MPSi and	l DEPSi
----------	----------------	----------------	-------------------	---------

Sample	Porosity from $N_2$ adsorption	Porosity from FFT	Refractive index
MPSi	63%	65%	1.62
DEPSi	73%	70%	1.5

of interference due to additional optical path caused by bacteria (see waveform sketch right from detector on Fig. 5).

The data from detector of DEPSi-based sensor element are shown on Figure 5a. There are interference spectra for the sample before adhesion of bacteria (black curve), after adhesion of the bacteria (red curve) and after further removal of the bacteria by an ethanol solution (blue curve). One can notice from the spectra significant shift of maxima for red curve, which is easier to notice on the right side of the spectrum. Then after rinsing of the bacteria maxima return to initial position. For better quantitative characterization of interferential parameters fast Fourier transform (FFT) of the spectra has been performed. The result is shown on Figure 5b with the same color encoding. Here one can see not only shift of the position of maximum (about 2%), which corresponds to increase of period of interference, but also significant broadening of the band up to 30%. The broadening is reversible and it arises only, when some bacteria are adsorbed on the DEPSi surface.

FFT combined with Bruggeman effective medium approach can give us values of porosity and effective refractive index,  $n_{eff}$ . Taking into account two component medium consisted of pores ( $n_{air} = 1$ ) and silicon ( $n_{Si} = 3.4$ ) the following values shown in Table I have been obtained:

#### Conclusions

Thus, new method of formation of double-layered porous silicon structures based on combination of electrochemical etching of silicon wafers and metal-assisted chemical etching has been proposed. Surface of the structure demonstrate hydrophilic properties, what is good for interaction with bacteria. According to scanning electron microscopy images top layer has increased roughness and a lot of cavities and traps for bacteria. Nevertheless, the optical quality of the structure was still high enough to measure Fabry-Perot interferometric infrared spectra, which were reversely modified by adhesion of E. Coli. Those interferograms have been processed via fast Fourier transform, and sufficient shift and broadening after adhesion have been demonstrated. Sensitivity of proposed sensor element can be estimated as  $10^4$  CFU per mL taking into account big 30% broadening of the line for  $10^5$  CFU per mL. Efficacy of the adhesion was additionally confirmed by FTIR measurements, which demonstrated presence of C=C and C=C groups typical for bacteria. Obtained results can be used for development of industrial optical sensors for airborne and water pathogens.

## Acknowledgments

This scientific research was supported by the Foundation for the Promotion of Small Innovative Enterprises in the Scientific and Technical Sphere under the UMNIK program on the topic of "Development of a sensor for bacteria based on silicon nanowires" under the contract No. 9744GU2/2015 from 15.02.2016 and by Russian Science Foundation (RSF) grant No. 17-12-01386. Authors are grateful to Dr. E. Scheval for assistance with bacteria experiments.

#### References

- G. Zheng, F. Patolsky, Y. Cui, W. U. Wang, and C. M. Lieber, *Nat. Biotechnol.*, 23, 1294 (2005).
- 2. I. Baltog, M. Baibarac, and S. Lefrant, J. Opt. A Pure Appl. Opt., 7, 632 (2005).
- 3. M. S. Mannoor et al., *Nat. Commun.*, **3**, 763 (2012).
- 4. A. Janshoff et al., J. Am. Chem. Soc., 120, 12108 (1998).
- 5. M. J. Sailor and J. R. Link, Chem. Commun., 1375 (2005).
- 6. L. T. Canham, Appl. Phys. Lett., 57, 1046 (1990).
- 7. a. G. Cullis, L. T. Canham, and D. J. Calcott, J. Appl. Phys., 82, 909 (1997).
- A. Jane, R. Dronov, A. Hodges, and N. H. Voelcker, *Trends Biotechnol.*, 27, 230 (2009).
- 9. E. Tenenbaum and E. Segal, Analyst, 140, 7726 (2015).
- 10. S. D. Alvarez et al., phys. stat. sol., 207, 1439 (2007).
- 11. Y. Mirsky et al., *Appl. Phys. Lett.*, **103**, 33702 (2013).
- 12. N. Massad-Ivanir, G. Shtenberg, and E. Segal, in, p. 37, Springer Netherlands (2012).
- 13. Selena Chan, S. R. Horner, P. M. Fauchet, and B. L. Miller, (2001).
- V. S. -Y. Lin, K. Motesharei, K. -P. S. Dancil, M. J. Sailor, and M. R. Ghadiri, *Science*, 278 (1997).
- Keiki-Pua S. Dancil, A. Douglas, P. Greiner, and M. J. Sailor, *J. Am. Chem. Soc.*, 121, 7925 (1999).
- 16. C. Pacholski et al., J. Am. Chem. Soc., 127, 11636 (2005).
- 17. O. Fellahi et al., Nanotechnology, 24, 495101 (2013).
- 18. M. Lv et al., Adv. Mater., 22, 5463 (2010).
- 19. L. A. Osminkina et al., Nanoscale Res. Lett., 7, 524 (2012).
- 20. X. Li et al., *Electrochim. Acta*, **138**, 476 (2014).
- 21. X. Li, Curr. Opin. Solid State Mater. Sci., 16, 71 (2012)
- 22. L. A. Osminkina et al., Nanoscale Res. Lett., 9, 463 (2014).